



Seasonal Enzyme Activities of Sympatric *Calanus glacialis* and *C. finmarchicus* in the High-Arctic

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In the Arctic shelf seas, the mesozooplankton biomass is dominated by the arctic copepod *Calanus glacialis*, but its boreal congeneric *C. finmarchicus* is expanding northwards. Even though it is already there, *C. finmarchicus* may not be able to truly establish itself in the Arctic seas and potentially replace *C. glacialis*. We compared metabolic and digestive enzyme activities of sympatric *C. glacialis* and *C. finmarchicus* from Isfjorden, Svalbard and off-shelf north of Svalbard. The seasonal regulation of anabolic and catabolic enzyme activities was generally similar for the two species, but with some interspecific differences corresponding to their ontogeny. Wake-up from overwintering started earlier in adults of *C. glacialis* than in *C. finmarchicus*, while the onset of dormancy started early in the overwintering stages of both species. Furthermore, *C. glacialis* showed an earlier and higher mobilization of lipase enzyme activities, indicating higher efficiency in assimilating dietary lipids compared to *C. finmarchicus*. Similar population sizes and population structures for *C. finmarchicus* off-shelf north of Svalbard and in Isfjorden support a similar origin. Still, *C. finmarchicus* was able to match regulation of enzyme activities to the bloom even though the bloom peaked approximately a month later off-shelf north of Svalbard, indicating that food availability is an important signal for the final step of termination of diapause. Even though the two species largely follow the same patterns of metabolic enzyme activities, the more efficient lipid anabolism of *C. glacialis* may give it an advantage over *C. finmarchicus* in high-Arctic unpredictable environments with short-pulsed primary production regimes.

Keywords: calanoid copepods, overwintering, metabolism, enzyme activities, Svalbard

1 INTRODUCTION

Calanoid copepods of the genus *Calanus* dominate the mesozooplankton community in Arctic and sub-Arctic seas in terms of biomass (>60%) (Jaschnov, 1970; Blachowiak-Samolyk et al., 2008; Kosobokova et al., 2011) and constitute a key link between primary producers and higher trophic levels (Falk-Petersen et al., 2007). In large calanoid copepods, lipids may comprise up to 70% of their dry weight (Sargent and Falk-Petersen, 1988; Lee et al., 2006), which they acquire mainly during

short periods of primary production lasting from two to eight weeks at high latitudes (reviewed by Leu et al., 2015). The conversion of their food to energy-rich lipids is key to the rapid and efficient transfer of energy and essential fatty acids from primary producers to higher trophic levels in arctic marine ecosystems (Falk-Petersen et al., 1990; Dahl et al., 2003). For the copepods, the accumulated lipid stores are necessary to fuel molting, gonad maturation and egg production in the following season (Falk-Petersen et al., 2009; Banas et al., 2016). In the most extreme environment – the deep Arctic Ocean – these copepods must be capable of surviving up to 10–11 months without food. The lack of sun during the 4-month polar night as well as the ice cover may efficiently block the incoming solar light into the summer. They also utilize the stored lipids for their basic metabolism during the extended periods without food through the arctic autumn and winter (Falk-Petersen et al., 2009). To preserve the lipids in this period, *Calanus* spp. perform seasonal vertical migrations, descending to depth and reducing their metabolism to a minimum when winter approaches, a physiological state named diapause or dormancy (Hirche, 1996; Freese et al., 2017).

Three species of the genus *Calanus* co-occur in the European Arctic: the two arctic species *Calanus hyperboreus* and *C. glacialis*, and the North Atlantic *C. finmarchicus* (Conover, 1988; Choquet et al., 2017), of which the two latter are the focus in this study. *Calanus glacialis* is considered an arctic species, though isolated populations have been identified along the Norwegian coast (Choquet et al., 2017). It has a 1–2-year life cycle and exhibits a mixed breeding strategy, meaning that it can mature and produce eggs at low rates, likely fueled by internal resources (capital breeding) early in the season, and then egg production rates increase when food becomes available with the bloom (income breeding, Hirche and Kattner, 1993). The boreal *C. finmarchicus*, which is smaller than *C. glacialis*, has a 1-year life cycle at its northernmost distribution and is considered to be an income breeder (Plourde and Runge, 1993; Kjellerup et al., 2012). Their difference in life cycle is also reflected in their overwintering stages. *Calanus glacialis* overwinters from CIV on and *C. finmarchicus* overwinters primarily as CV (Falk-Petersen et al., 2009).

Diapause is controlled endogenously and is a physiological reaction to reoccurring adverse environmental conditions such as low food availability during winter (Dahms, 1995). Diapause is commonly performed by specific ontogenetic stages in crustaceans (Guppy and Withers, 1999), such as the overwintering stages in *Calanus* spp. (see above). Five phases of diapause have been described in copepods: 1) the preparatory phase when the organisms accumulate lipids (energy reserves) and arrest further development and growth, 2) the induction phase when the organisms stop feeding and reduce their metabolism, 3) the refractory phase when the organisms are torpid and reach minimum metabolic activity, 4) the activation phase when organisms regain their ability to develop, and, finally 5) the termination phase when the organisms reach full metabolic activity again (Hirche, 1996). Depending on environment, latitude and season, dormancy strategies and

ontogenetic migrations may vary profoundly among species and even within populations of the same species (Hirche, 1996; Hirche, 1998; Darnis and Fortier, 2014).

In the Svalbard-Barents Sea region, *C. finmarchicus* and *C. glacialis* are regarded as important beacons of climate change (Wassmann et al., 2015). A higher proportion of *C. finmarchicus* compared to *C. glacialis* reflects a strong influence of Atlantic water and a relatively warm arctic climate while the opposite indicates a colder arctic climate. A substantial borealization of the arctic zooplankton community is predicted with global warming (Hays et al., 2005). In the surrounding path of the North Atlantic Current into the Fram Strait and the Barents Sea, an increase in abundances of the boreal *C. finmarchicus* and other North Atlantic species has already been recorded (Wassmann et al., 2006; Aarflot et al., 2017; Hop et al., 2019a; Hop et al., 2019b). To better predict the consequences of the rapid warming for these key species in the Arctic, better knowledge on physiological adaptations of zooplankton species is needed. Baseline information on the natural seasonal variability in metabolism is important in order to identify physiological responses directly linked to the ongoing climate change (e.g., Møller et al., 2016).

Seasonal changes in metabolic activity can be determined by measuring the relative activity level of enzymes of central metabolic pathways (e.g., Freese et al., 2017). For instance, citrate synthase (CS) and malate dehydrogenase (MDH), which catalyze different reactions in the citric acid cycle, are regarded as good indicators of the aerobic potential of an organism and thus its overall metabolism (Torres and Somero, 1988; Meyer et al., 2002; Teschke et al., 2007). MDH is strongly correlated with respiration (Meyer et al., 2010) and the advantage of measuring MDH rather than respiration by incubations is the minimum laboratory handling effect (Ohman et al., 1998). CS on the other hand has also been found to correlate with egg production (Kreibich et al., 2008). Catabolism of stored lipids, which occurs mainly in January–March for *C. glacialis* (Freese et al., 2017), is positively correlated to activity of 3-hydroxyacyl-CoA dehydrogenase (HOAD), a key enzyme of the β -oxidation of fatty acids (Auerswald and Gäde, 1999; Hassett, 2006). Further, the HOAD activity is inversely related to digestive enzyme activities (Freese et al., 2016; Freese et al., 2017). The major components of the copepods' diet are proteins and lipids, which are processed by proteinase and lipase/esterase, respectively. Peak digestive enzyme activities in *C. glacialis* are found when algal food is plentiful and then gradually decrease throughout the autumn–winter (Freese et al., 2016). Overall, the metabolism of *C. glacialis* is reduced to half during winter, which suggests that this species is not going into a proper diapause as defined by Hirche (1996) (Freese et al., 2017). However, respiration rates of both species in winter have been found to be reduced by a similar amount [40–65%; *C. finmarchicus* Hirche (1983); *C. glacialis*: Morata and Søreide (2015)] and do not suggest large inter-species differences in the overwintering metabolic states. How *Calanus* spp. regulate their physiology to best schedule their complex life history to the strong seasonality at high latitudes is still poorly understood.

In this study, we were particularly interested in the timing of physiological changes in co-occurring *C. glacialis* and *C. finmarchicus*, and how such changes are related to life history strategies and the environmental conditions. Possible interspecies differences may provide new insights into why *C. glacialis* is specially adapted to the Arctic while *C. finmarchicus* thrives under more temperate North Atlantic conditions.

We performed monthly sampling of *C. glacialis* and *C. finmarchicus* over a 15-month period in Isfjorden, a high-Arctic (78°N) fjord in Svalbard with relatively high water temperatures (>1°C) and no seasonal ice. Samples were collected to assess general metabolism (CS and MDH), digestion of incoming food (proteinase and lipase) and utilization of stored lipid resources (HOAD). For comparison, we also collected samples off-shelf north of Svalbard. For comparing the timing of changes in co-occurring *C. glacialis* and *C. finmarchicus*, our major research questions were (1) Do the metabolic and catabolic enzyme activities follow the same seasonal patterns in the two *Calanus* species? (2) Do the seasonal patterns in digestive enzyme activity differ between the species? And (3) How do enzyme activity patterns compare between off-shelf and fjord individuals?

2 MATERIAL AND METHODS

2.1 Sampling Area and Sample Processing

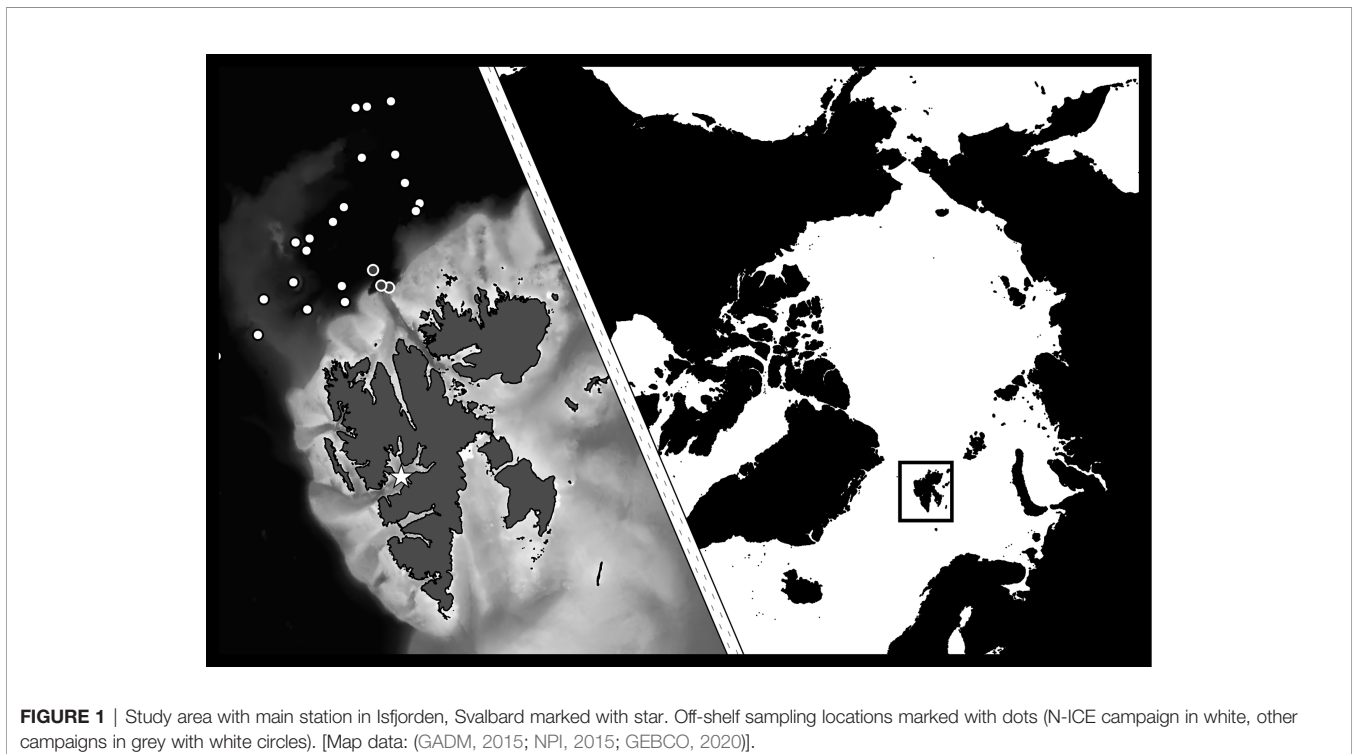
Calanus glacialis and *C. finmarchicus* were collected monthly from June 2015 to August 2016 at Karlskronadupet (78°19'N; 015°10'E) (**Figure 1**), a 274 m deep basin in the central part of the Isfjorden system, Svalbard. The mouth of Isfjorden is open

towards the shelf and slope area along west Spitsbergen, with no sill limiting the inflow of Atlantic water *via* the West Spitsbergen Current (WSC) and of Arctic water *via* the East Spitsbergen Current. Both these currents are flowing from south to north along the shelf break and shelf respectively (Nilssen et al., 2008).

Samples were also collected off-shelf north of Svalbard (**Figure 1** and **Supplementary Table S1**). The main sampling period off-shelf north of Svalbard was from February to June during the N-ICE 2015 campaign when RV *Lance* was frozen into the ice (Granskog et al., 2018), and additional off-shelf sampling north of Svalbard was conducted with RV *Helmer Hanssen* in late summer 2015 and 2016.

Sampling in Isfjorden was conducted from larger vessels (RV *Helmer Hanssen*, KV *Svalbard*, RV *Dalnie Zelentsy*) and smaller boats (RV *Viking Explorer* and *UNIS Polaris*). Data on water column properties were collected using a SAIV SD204 CTD or an SBE Seabird Electronics CTD; and identification of water masses was based on Skogseth et al. (2020) for the fjord station, and on Meyer et al. (2017) and Rudels et al. (2000) for off-shelf north of Svalbard. Data from fluorometers attached to the CTDs were used to estimate the average Chlorophyll *a* (Chl *a*) concentration corresponding to the depth intervals where zooplankton was sampled (see below). The fluorescence sensor data were verified by comparison with Chl *a* measured fluorometrically. Water samples were collected at several depths and filtered on GF/F filters. The samples were extracted in methanol for 24 h at 4°C before Chl *a* concentration was measured with an AU10 Turner Fluorometer (Turner Design, Inc.).

Stratified zooplankton samples were collected with a MultiNet Midi (0.250 m² opening, mesh size 200 μm, Hydro-Bios) or a WP2



closing net (0.255 m² opening, mesh size 200 µm, Hydro-Bios). Copepods for enzyme analyses were collected from those layers in which the majority of the population resided. On 15 January 2016, off-shelf, and 4 April 2016, in Isfjorden, samples were collected both from surface and depth (**Table S1**). Sorting was conducted either immediately after sampling on board or within 12 h after sampling at return to the University Centre in Svalbard (UNIS). Previous studies on calanoid copepods have showed that it takes at least a week for changes to occur in digestive (Landry, 1983; Head and Conover, 1983) and metabolic (Freese, 2015) enzyme activities when conditions are altered, thus the effect of the up to 12-hour delay should be negligible. In either case, sorting was conducted in a temperature-controlled room at 2–4°C to avoid temperature stress on the copepods. The dominating stages of *C. glacialis* and *C. finmarchicus* were sorted in triplicates à 10 and 15 individuals for enzyme activity analyses and single individuals of the same stages were collected in tin caps to determine dry mass (**Supplementary Table S1**). Individuals were quickly rinsed in distilled water, blotted briefly by touching them to a tissue paper and transferred to cryo vials before being snap frozen in liquid nitrogen. Samples were stored at -80°C and transported to the Alfred Wegener Institute, Bremerhaven, Germany, either in a dry shipper or on dry ice for further analyses.

2.2 Morphological Identification of *Calanus* spp.

The different species of *Calanus* were initially distinguished, while alive, by their prosome length according to Daase and Eiane (2007) with adjustments made for CV and adult females after Gabrielsen et al. (2012), as presented in Daase et al. (2018). From copepodite stage CIV and older, *C. hyperboreus* was also distinguished by the presence of an acute spine on the fifth thoracic segment. Pigmentation was used as an additional indicator of alive specimens since *C. glacialis*, from these regions, tend to have more red pigmentation on the antennules and genital segment of the urosome (females only) compared to *C. finmarchicus* (Nielsen et al., 2014; Choquet et al., 2018; Trudnowska et al., 2020).

2.3 Analyses of Enzyme Activity

Enzyme activities in both *C. glacialis* and *C. finmarchicus* were measured at the Alfred Wegener Institute in Bremerhaven, Germany. Measurements were conducted in triplicates for two digestive enzymes (proteinase and lipase/esterase), and for three metabolic enzymes [Malate dehydrogenase (MDH), Citrate synthase (CS), and 3-Hydroxyacyl-CoA dehydrogenase (HOAD)]. Proteinase, lipase/esterase and CS activities were all measured in the same extract of 10 individuals, from the sorted triplicates à 10 individuals (section 2.1). Individuals for MDH and HOAD measurements were collected from each of the triplicates à 15 individuals. Two of these were pooled and extracted for MDH activity analyzes, three of these were pooled and extracted for HOAD activity, and the remaining 10 in each vial were kept as back up.

2.3.1 Metabolic Enzymes

2.3.1.1 Malate Dehydrogenase (MDH)

Measurements of the Malate dehydrogenase activity (EC 1.1.1.37) were modified after Teschke et al. (2007) as described

in Freese et al. (2017). The frozen copepod samples were transferred to 1.5 ml Eppendorf tubes in triplicates of two individuals each and homogenized by hand with a micropestle in 40 µl 0.1 M potassium phosphate buffer at pH 7.0. The homogenates were centrifuged for 15 min at 15,000 g at 4°C and the liquid phase was transferred to a new 1.5 ml Eppendorf tube. The sample was diluted 1:10 by adding 6 µl of the sample to a vial with 54 µl of buffer. Measurements of enzyme activity were done in a 96 well plate. Buffer (180 µl), 6.7 µl NADH (Roche Diagnostics 10107735001) and 6.7 µl sample were mixed and incubated at 25°C for 5 min. The reaction was started by adding 6.7 µl oxaloacetic acid and the absorbance was measured for 5 min at 25°C and 340 nm in a Synergy HTX Multi-Mode Reader using the software KC4 3.4 Rev.21. The slope of the curve from 1–4 min was used to calculate the enzyme activity.

2.3.1.2 Citrate Synthase (CS)

Citrate synthase activity (EC 4.1.3.7.) was measured after Stitt (1984) and Saborowski et al. (2002), but with a different buffer system. Enzymes were extracted from the frozen copepods as described below (see “Digestive Enzymes”). In a semi-microcuvette, 20 µL 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma-Aldrich, D8130), 20 µL Acetyl-CoA (Acetyl-Coenzyme A trilithium salt, Roche Diagnostics, 13893324), 20 µL sample extract, or buffer for controls, and 520 µL 0.1 M Tris/HCl (supplemented with 10 mM CaCl₂) buffer at pH 7.0 were mixed. The mixture was pre-incubated in the spectrophotometer (Thermo Scientific, UV1), kept at 25°C using a Peltier element (Krüss Optronic), for 5 min before 20 µL oxaloacetic acid (Sigma-Aldrich, O4126) was added to start the reaction. The absorbance was measured regularly in the spectrophotometer for 3 min at 405 nm, and the measurements were recorded with the software VisionLite (version 2.2).

2.3.1.3 3-Hydroxyacyl-CoA Dehydrogenase (HOAD)

Measurements of 3-Hydroxyacyl-CoA dehydrogenase activity (EC 1.1.1.35) were modified after Auerswald and Gäde (1999) as described in Freese et al. (2017). The frozen copepod samples were transferred to 1.5 ml micro centrifuge tubes in triplicates à three individuals and homogenized by hand with a micropestle in 180 µl 107 mM triethanolamine/HCl buffer (supplemented with 5.3 mM EDTA) at pH 7.0. The homogenates were centrifuged at 15 000 g at 4°C for 15 min and the liquid phase was transferred to a new 1.5 ml Eppendorf tube. Measurements of enzyme activity were done in a 96 well plate. 180 µl buffer, 6.7 µl NADH (see above) and 6.7 µl sample were mixed and incubated at 25°C for 5 min. The reaction was started by adding 6.7 µl Acetoacetyl-CoA (Sigma A-1625) and the absorbance was measured for 8 min at 25°C and 340 nm in a Synergy HTX Multi-Mode Reader using the software KC4 3.4 Rev.21. The slope of the curve from 3–7 min was used to calculate the enzyme activity. Unfortunately, we experienced technical difficulties when measuring HOAD in *C. glacialis* from winter and thus were not able to obtain consistent HOAD measurements throughout the year.

2.3.2 Digestive Enzymes

The frozen copepod samples were transferred to 1.5 ml Eppendorf tubes in triplicates à 10 individuals and

homogenized by hand with a micropestle in 200 μL 0.1 M Tris/HCl (supplemented with 10 mM CaCl_2) buffer at pH 7.0. The homogenates were centrifuged at 15 000 g at 4°C for 15 min. The liquid phase was transferred to a new 1.5 ml Eppendorf tube and the remaining tissue was stored at -80°C for later molecular verification of species. This extract was used for measurements of proteinase, lipase/esterase and CS.

2.3.2.1 Proteinase Activity

Total proteinase activity (EC 3.4.21-24) was measured after Saborowski et al. (2004), modified after Kreibich et al. (2008). Sample (20 μL), or buffer for controls, was pipetted into 1.5 ml Eppendorf tubes and pre-incubated at 30°C for 5 min on a thermo shaker at 400 rpm. To each vial, five μL azocasein (1% in deionized water, Fluka BioChemika, 11615) was added before incubation for another 60 min. The reaction was stopped by adding 50 μL trichloroacetic acid (TCA, 8%) and the vials were centrifuged at 15 000 g at 4°C for 15 min. The supernatant was transferred to an ultra-microcuvette (Hellma 105.203-QS). The optical density of the supernatant was measured with a spectrophotometer (Thermo Scientific, UV1) at 366 nm (dE_{366}) and recorded with the software VisionLite (version 2.2).

2.3.2.2 Lipase/Esterase Activity

Lipase/esterase activity (EC 3.1.1.) was measured after Knotz et al. (2006). Sample (20 μL), or buffer for controls, was diluted in 470 μL 0.1 M Tris/HCl (supplemented with 10 mM CaCl_2) buffer at pH 7.0. Ten μL 4-methylumbelliferyl butyrate dissolved in dimethyl sulfoxide (5 mmol L^{-1} , MUF-butyrate, Fluka BioChemika, 19362; DMSO, AppliChem A3608) were added to each vial and incubated in the dark at 25°C for 30 min on a thermo shaker at 400 rpm. Fluorescence was measured with a NanoDrop 3300 at 360 nm (excitation) and 450 nm (emission) and recorded with the software ND-3300 V 2.7.0.

2.4 Molecular Identification of Species

Because of the uncertainty of differentiating between *C. glacialis* and *C. finmarchicus* based on morphology (Choquet et al., 2018), the remaining tissue pellets after the first batch of enzyme extraction were kept and stored at -80°C for molecular species identification. The pellets were transported in a dry shipper to Nord University in Bodø (Norway) where molecular analyses were conducted.

The species composition of the tissue pellets, i.e., from pooled individuals, was genetically assessed by analysis of species-specific profiles of length polymorphism in six nuclear molecular markers characterized by motifs of insertion or deletion (i.e., InDels) (Smolina et al., 2014), following Choquet et al. (2017). In short, DNA was extracted from the remaining pellets of *Calanus* tissue, following the HotSHOT DNA extraction method (Montero-Pau et al., 2008). Six InDel markers were amplified in a multiplexed Polymerase Chain Reaction (PCR). The resulting amplified markers were sized on a 3500xL Genetic Analyzer (Applied Biosystems), generating either a “pure” species-specific profile characteristic of *Calanus finmarchicus* or *C. glacialis* (see Smolina et al., 2014) when only one species was present within a pool, or a profile containing

mixed signals from both species when two species were present within a pool.

2.5 Dry Mass and Carbon and Nitrogen Content

At each sampling, 24 individuals of both *C. glacialis* and *C. finmarchicus* were collected for dry mass (DM) and Carbon and Nitrogen (CN) analyses. The animals were rinsed quickly in distilled water before being placed individually in pre-weighed tin capsules (IVA analysentechnik, SA76981102) and dried at 60°C for 24 h. DM was determined by weighing the samples in the tin capsules and subtracting the pre-weight. After weighing, the tin capsules were packed tight and the samples were analyzed for CN content with an element analyzer (vario EL cube, Elementar).

2.6 Weighted Mean Depth

Weighted mean depth (WMD) was calculated for the total population of *C. glacialis* and *C. finmarchicus* following equation 1:

$$\frac{\sum_{i=1}^n (a_i d_i) D_i}{\sum_{i=1}^n a_i d_i} \quad (1)$$

Where a_i is the number of individuals per m^3 of species a in depth stratum i , d_i is the sampled distance in depth stratum i , D is average depth in depth stratum i and n is the number of depth strata at a station.

2.7 Statistical Analysis

Statistical analyses were done in Sigmaplot (14.0, Systat Software, San Jose, CA). T-tests were applied to investigate potential differences in enzyme activities among species, stages and dates. Correlations between changes in enzyme activities and physical (temperature, light) and biological (Chl a , Weighted Mean Depth (WMD) of the community) factors, as well as among enzyme activities, were done with Pearson correlations when linear relationships and normally distributed data occurred, and Spearman’s rank-order correlation when not. The significance level was set to $\alpha=0.05$ in all tests.

3 RESULTS

3.1 Environmental Conditions

3.1.1 Chlorophyll a

The fluorescence sensor data, as verified with Chl a data measured fluorometrically and described in detail by Eide (2016) for Isfjorden and Assmy et al. (2017) for off-shelf area north of Svalbard, captured well the seasonality in Chl a concentrations.

In Isfjorden, the spring bloom had commenced ($1.5 \mu\text{g Chl } a \text{ L}^{-1}$) at the start of the sampling campaign in June 2015. Relatively high Chl a concentrations persisted throughout July and August (2.2 and $2.4 \mu\text{g L}^{-1}$, respectively; **Figure 2**). From October 2015 to April 2016, typically low winter Chl a values ($< 0.01 \mu\text{g Chl } a \text{ L}^{-1}$) were found. The first increase was recorded in early May ($2.1 \mu\text{g Chl } a \text{ L}^{-1}$), and peak Chl a concentrations were found between mid-May and early June 2016 ($> 7.0 \mu\text{g Chl } a \text{ L}^{-1}$). In July 2016, Chl a concentrations were found to be in the same range as in the previous summer ($2.1 \mu\text{g Chl } a \text{ L}^{-1}$).

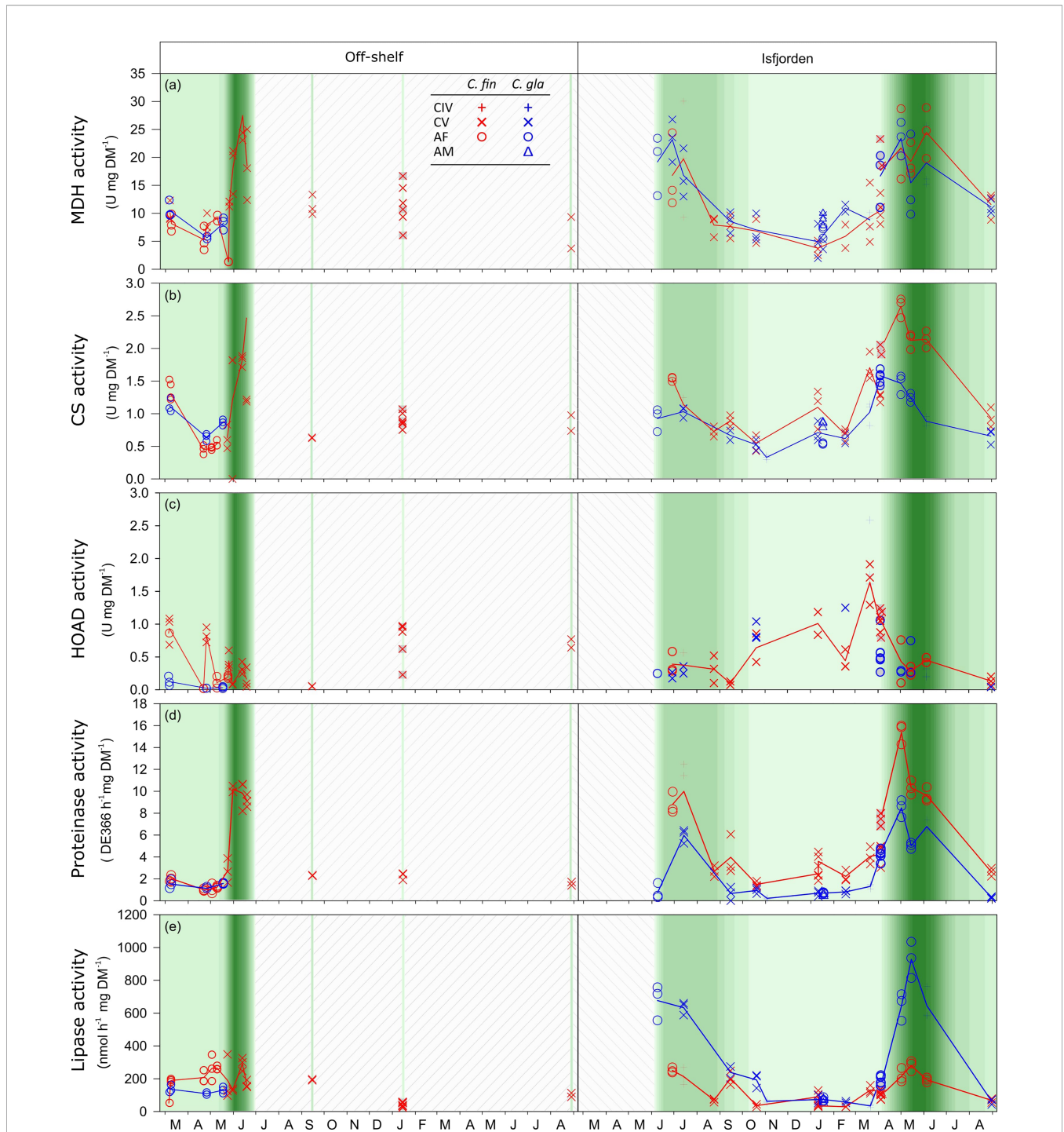


FIGURE 2 | Enzyme activities for *Calanus finmarchicus* (red) and *C. glacialis* (blue). Data from off-shelf are shown in the left panels and data from Isfjorden are shown in the right panels. The background presents data from the Chl *a* max ranging from <0.01 (lightest green) to 8 $\mu\text{g Chl a L}^{-1}$ (darkest green). White background with diagonal lines means no data on Chl *a* max. Specific activities are indicated for **(A)** Malate dehydrogenase, **(B)** Citrate synthase, **(C)** 3-hydroxyacyl-CoA dehydrogenase (HOAD), **(D)** protease, and **(E)** lipase. Each point represents one measurement, and the line connects the averaged levels of activity. For dates with sampling both in the surface and at depth, the surface samples are highlighted with grey fill.

$a L^{-1}$). In late August, the Chl a concentration dropped to $0.73 \mu g Chl a L^{-1}$ (Figure 2). Off-shelf north of Svalbard, low winter algal biomasses ($< 0.01 \mu g Chl a L^{-1}$) prevailed until mid-May in 2015 during the N-ICE drift campaign (Figure 2). Peak phytoplankton bloom concentrations ($8 \mu g Chl a L^{-1}$) were recorded in early June and remained relatively high ($4.7\text{--}6.3 \mu g L^{-1}$) until late June when the last measurement was done ($2.4 \mu g L^{-1}$, Figure 2). North of Svalbard in September 2015 and August 2016, the Chl a concentrations were elevated in the upper 20 m with respective $1.4 \mu g Chl a L^{-1}$ (15 m) and $1.3 \mu g Chl a L^{-1}$ (14 m). In January 2016, no fluorescence was detectable (Figure 2).

3.1.2 Hydrography

In Isfjorden during the sampling period, the water column was mostly dominated by transformed Atlantic water. However, stratification was observed during both summers with warmer ($> 4^{\circ}C$) and less saline (< 33) Surface Water in the upper ~ 25 m compared to the waters below. There was also an influx of Atlantic water in December 2015, observed as an increase in both temperature and salinity at around 100 m depth (Figure 3).

Off-shelf north of Svalbard during the drift with RV *Lance* in the N-ICE campaign, we passed over the Yermak Plateau, an area influenced by the north-flowing West Spitsbergen Current (Meyer et al., 2017). In the consolidated pack ice north of Svalbard, the hydrography was characterized by a cold ($< 0^{\circ}C$) relatively fresh (< 34.5) and deep mixed surface layer of Polar Surface Water down to 100 m, and warmer ($> 2^{\circ}C$) and more saline (> 34.5) Atlantic Water and Modified Atlantic Water were located between 100–500 m (Meyer et al., 2017). Hydrographic conditions changed significantly after 25 May, when the Atlantic Water was found closer to the surface and the mixed surface layer became thinner, fresher, and warmer (Figure 3).

3.2 Success of Morphological Species Identification for Enzyme Samples

After extraction of enzymes from the samples á 10 individuals (section 2.3.2), the remaining tissue pellets provided sufficient material for molecular identification (section 2.4). 65% of these tissue pellets were sequenced, with resulting success rate of morphological identification at 97% for Isfjorden and at 85% for the off-shelf stations. The enzyme measurements from the six extracts of mixed samples were discarded. In total, 49 extracts were not tested because of a shipping issue; 29 from Isfjorden and 20 from off-shelf. Applying the same success rate of identification as the tested samples indicate that among the untested extracts, one from Isfjorden and three from off-shelf may be mixed. Based on the high success rate of the tested samples and the fact that each sample is made up of several individuals that will mitigate the impact of the occasional misidentified individual, these samples and two extracts with inconclusive molecular ID were treated as correctly identified in further analyses. The samples of mixed ID, inconclusive and not tested are listed in Table S2.

3.3 Enzyme Activities

3.3.1 Metabolic Enzyme Activities

The MDH and CS activities followed the same seasonal trend for both species in Isfjorden, with high levels in spring and summer and low levels in autumn and winter (Figure 2). For both enzymes, the specific activity (activity $mg DM^{-1}$) was low prior to the bloom and increased rapidly for a short period, peaking in mid-June (Figure 2).

The timing of peak MDH activity and the CS activity levels differed between the two species. For MDH, the specific activities in the two species were in the same range. However, peak MDH activities were reached one month earlier in *C. glacialis* than in

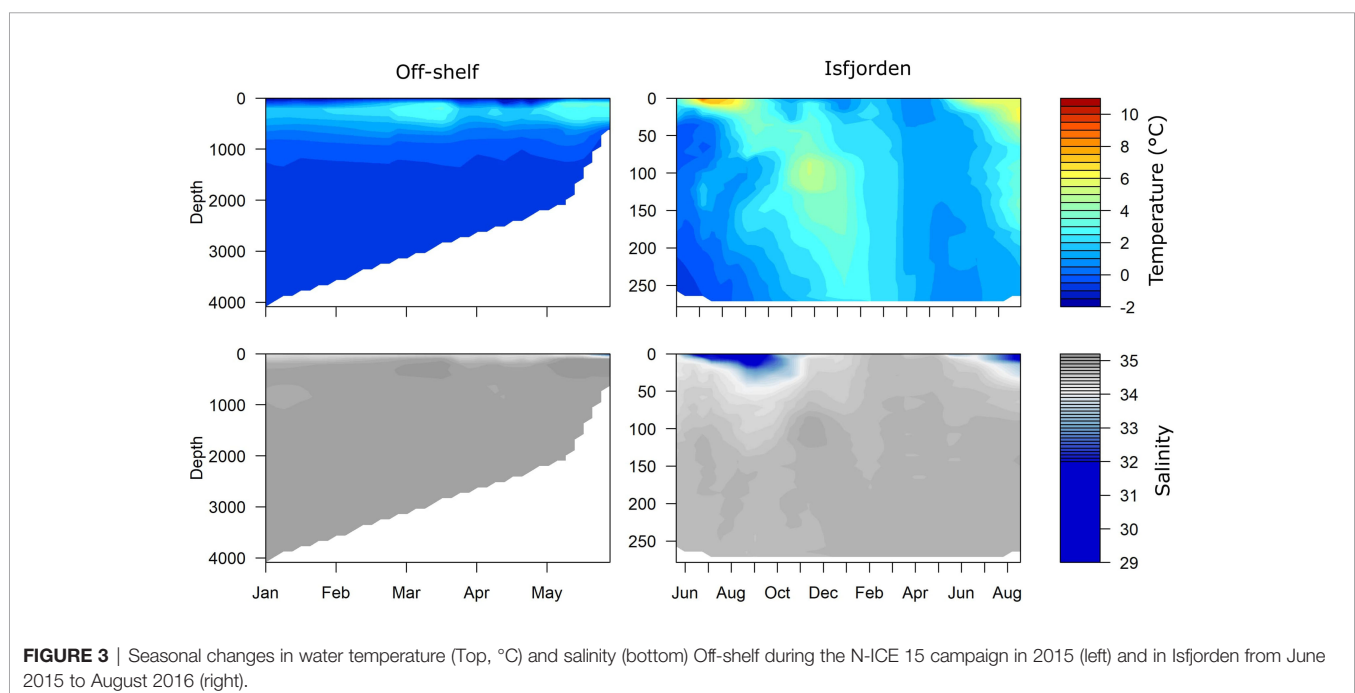


FIGURE 3 | Seasonal changes in water temperature (Top, $^{\circ}C$) and salinity (bottom) Off-shelf during the N-ICE 15 campaign in 2015 (left) and in Isfjorden from June 2015 to August 2016 (right).

C. finmarchicus (Figure 2A). Maximum MDH levels were recorded in early May (23.3 U mg DM⁻¹) in *C. glacialis* and June (24.4 U mg DM⁻¹) in *C. finmarchicus* (Figure 2A). For both species, minimum MDH activity levels were recorded in January (3.7–4.9 U mg DM⁻¹). For CS, *Calanus finmarchicus* had higher activity (2.6 U mg DM⁻¹) in spring-summer than *C. glacialis* (1.6 U mg DM⁻¹), while the CS activity levels decreased to similarly low levels (~1 U mg DM⁻¹) during autumn and winter for both species (Figure 2B). The CS and MDH activities in Isfjorden were strongly positively correlated to day length (as well as to Chl *a*) in *C. finmarchicus*, but not in *C. glacialis* (Table 1).

3.3.2 Catabolism of Body Lipids

Peak HOAD activity occurred in March in both *C. glacialis* (1.98 U mg DM⁻¹) and *C. finmarchicus* (1.63 U mg DM⁻¹). Minimum activities were measured in late August in *C. glacialis* (0.02 U mg DM⁻¹) and in mid-September in *C. finmarchicus* (0.08 U mg DM⁻¹) (Figure 2C). In *C. finmarchicus*, HOAD was negatively correlated (Pearson, *r* = -0.57, *p*<0.05) with Chl *a*, whereas no significant correlation was found for *C. glacialis* (Pearson, *r* = -0.04, Table 1).

3.3.3 Digestive Enzyme Activity

Strong positive correlations between Chl *a* concentration and digestive enzyme activities were found for both species (Table 1 and Figures 2D, E). In Isfjorden, the proteinase activity of *C. finmarchicus* varied between a minimum of 1.59 dE₃₆₆ h⁻¹ mg DM⁻¹ in October and maximum of 15.36 dE₃₆₆ h⁻¹ mg DM⁻¹ in early May (Figure 2D). In *C. glacialis* in Isfjorden, the highest proteinase activity was also observed in early May (8.47 dE₃₆₆ h⁻¹

mg DM⁻¹) and minimum proteinase activity was measured in early November with 0.2 dE₃₆₆ h⁻¹ mg DM⁻¹ (Figure 2D).

The lipase activity of *C. finmarchicus* was at its minimum in February (28.94 nmol h⁻¹ mg DM⁻¹) and its maximum in May (280.51 nmol h⁻¹ mg DM⁻¹). For *C. glacialis*, the minimum lipase activity was found in March (33.96 nmol h⁻¹ mg DM⁻¹) and maximum in May, as for *C. finmarchicus*, but with three times higher maximum lipase activities (927.22 nmol h⁻¹ mg DM⁻¹) than recorded for *C. finmarchicus* (*t*-test, *p*<0.001) (Figure 2E).

Off-shelf, a positive correlation, similarly as in Isfjorden, between the Chl *a* maximum and the activity of both proteinase and lipase was observed for *C. finmarchicus* (Table 1). Off-shelf proteinase activity of *C. finmarchicus* was at a minimum in early March (1.0 dE₃₆₆ h⁻¹ mg DM⁻¹) and increased to peak activity on May 31st (10.21 dE₃₆₆ h⁻¹ mg DM⁻¹). The lipase activity in off-shelf *C. finmarchicus* had a similar pattern as proteinase activities, with a minimum value measured in early March (189.65 dE₃₆₆ h⁻¹ mg DM⁻¹) and peak activities in mid-June (1458.98 dE₃₆₆ h⁻¹ mg DM⁻¹) (Figure 2). *Calanus glacialis* was only sampled during the pre-bloom, and at that time proteinase and lipase activities remained stable at 1.2–1.6 dE₃₆₆ h⁻¹ mg DM⁻¹ and 109.3–136.8 nmol h⁻¹ mg DM⁻¹, respectively (Figure 2).

4 DISCUSSION

4.1 Do *Calanus finmarchicus* and *C. glacialis* Metabolism Generally Follow the Same Seasonal Variability?

Both *C. glacialis* and *C. finmarchicus* followed similar and clear seasonal patterns of metabolic enzyme activities, with high

TABLE 1 | Correlation between the enzyme activity and the environmental factors Chlorophyll *a* max in the water column (Chl *a*), Weighted mean depth of community (WMD), temperature at weighted mean depth (WMD temp.) and Day length.

		CS	HOAD	Proteinase	Lipase	Chl <i>a</i>	WMD	WMD temp.	Day length
Isfjorden									
<i>Calanus finmarchicus</i>	MDH	0.674***	-0.120	0.768***	0.648***	0.618***	-0.637***	-0.498**	0.711***
	CS		0.278	0.854***	0.638***	0.329*	-0.647***	-0.539***	0.462**
	HOAD			-0.118	-0.174	-0.391*	0.177	0.090	-0.461**
	Proteinase				0.785***	0.571***	-0.803***	-0.601***	0.584***
	Lipase					0.647***	-0.652***	-0.545***	0.668***
<i>Calanus glacialis</i>	MDH	0.202	-0.304	0.714***	0.656***	0.433**	-0.676***	-0.775***	0.837***
	CS		0.007	0.386*	-0.015	0.304	-0.491**	-0.186	0.589***
	HOAD			-0.419*	-0.254	-0.103	0.496**	-0.058	-0.397*
	Proteinase				0.670***	0.544***	-0.898***	-0.576***	0.798***
	Lipase					0.797***	-0.757	-0.692***	0.774***
Off-shelf									
		CS	HOAD	Proteinase	Lipase	Chl <i>a</i>	Temperature	Day length	
<i>Calanus finmarchicus</i>	MDH	0.430*	-0.130	0.817***	0.34	0.623***	0.148	0.028	
	CS		-0.227	0.691***	0.231	0.537**	0.172	-0.222	
	HOAD			-0.148	-0.687***	-0.446**	0.275	-0.319	
	Proteinase				0.097	0.912***	0.124	-0.075	
	Lipase					0.161	-0.246	0.585***	

Correlations are calculated for all measurements done through the sampling year. Pearson correlation in normal font and Spearman in bold font, with *p*-value indicated as: *:*p*=0.05–0.01, **: *p*=0.01–0.001, ***: *p*<0.001.

activity in spring and summer and low activity during autumn and winter. In both species, MDH, a valid proxy for respiration and thus overall metabolism (Meyer et al., 2010), correlated well with day length, suggesting that light is an important factor regulating the seasonal pattern of the metabolic activity.

The seasonal pattern of metabolic enzyme activities also corresponded with the ontogenetic migration patterns of *Calanus* spp. (Figure S1; Hatlebakk et al., this issue). In Isfjorden, from which we have the longest (15 months) and most consistent data set, we found a negative correlation between MDH and WMD for both *C. glacialis* and *C. finmarchicus* supporting the assumption that *Calanus* spp. reduce their overall metabolism as they descend to depth (Hirche, 1996). Following the five phases of diapause described by Hirche (1996), we can assign the preparatory and induction phases of diapause to start in July-August corresponding to their seasonal descent. The refractory phase, i.e., when the lowest anabolic enzyme activities were recorded, occurred between September and January, followed by the activation phase in January-April, dependent on species, ontogenetic stage and presumably also body size (see below). The final termination phase was shorter and took place in April-May. In Isfjorden, minimum MDH activities, found in winter for both *C. glacialis* and *C. finmarchicus*, were 80% lower compared to specimen with maximum activity. In a previous study on *C. glacialis* in Billefjorden, Svalbard, Freese et al. (2017) showed a similar seasonal pattern, but their winter minimum activities were only 60% lower than summer maximum activities. Billefjorden is a colder, seasonally ice-covered branch of Isfjorden, and it is possible that differences in environmental conditions may have led to the differences in relative minimum enzyme activities while the general activity pattern was similar. It is, however, also possible that we missed the deepest state of dormancy since there was a gap in sampling between early November and mid-January (Figure 2B), and *C. glacialis* may reach the refractory phase only for a brief period (Varpe and Ejsmond, 2018). Also, the variability in MDH was high among the samples, and this may relate to differences in individual fitness (Hassett, 2006). In order to clarify whether environmental or physiological factors can explain differences in minimum activities in winter, sampling with a higher resolution would be necessary.

We still have a poor understanding of which external and internal cues trigger and regulate the metabolism of *Calanus*, and particularly the onset and termination of diapause. However, recent studies on the seasonal variability in ion concentration (Freese et al., 2015), enzymes (e.g., Freese et al., 2016; Freese et al., 2017) and gene expressions (Häfker et al., 2018), together with life history modelling (Banas et al., 2016; Ejsmond et al., 2018; Varpe and Ejsmond, 2018) have provided new insights into diapause regulation. While most studies assume that deep-dwelling copepods enter a dormant state (i.e., diapause), there does not seem to be a simple on-off switch for diapause (e.g., Häfker et al., 2018). Specimens are gradually lowering and increasing their metabolic rates throughout the year (Freese et al., 2017), which has been well described by Hirche (1996) when identifying the five different phases of diapause in

copepods. Diapause may be endogenously regulated (Häfker et al., 2018), or a gradual response to ceased feeding as suggested for *C. glacialis* by Freese et al. (2017), although it is most likely a complex combination of several factors.

There was an increase in abundance of adults over a period of 2-3 months for both species, but they appeared roughly two months earlier in *C. glacialis* than in *C. finmarchicus*, which confirms that *C. glacialis* reproduces earlier than *C. finmarchicus*, (Hatlebakk et al., this issue). This was further supported by an earlier peak of abundance in both *C. glacialis* eggs and young copepodite stages (CI-CIII, Hatlebakk et al., 2022). Gonad maturation is even more energy demanding than egg production (Jónasdóttir, 1999). In accordance, particularly high CS activities was found in *C. glacialis* males in January likely suggesting sperm formation. At that time, the male:female sex ratio was relatively high (0.4) and 9.2% of the females carried spermatophores (Daase et al., 2018), supporting that January was the time of male gonad maturation and mating.

The increase in metabolic enzyme activity would be expected to be coupled with increased HOAD activity, indicating utilization of stored lipid resources since external food is scarce in January. Accordingly, Freese et al. (2017) found the highest HOAD activity during mid-winter and decreasing activities when ice algae became present from mid-March on, providing food early in the season (Freese et al., 2016). We also found some indication of higher HOAD activity in CV *C. glacialis* in winter as compared to summer, but unfortunately, we experienced technical difficulties when measuring HOAD activities. In the period of low food availability, we were only able to obtain measurements of HOAD in October and one measurement in February and, thus, we are lacking data from mid-winter. However, HOAD activity was higher at these times compared to measurements for the spring and summer, and thus it is fair to assume the HOAD activity was increased in mid-winter.

In *C. finmarchicus*, the HOAD activity during winter was elevated compared to summer, and reached the highest levels just prior to the spring bloom. The relative abundance of adult *C. finmarchicus* also increased in March-April indicating molting and wake up from dormancy (Hatlebakk et al., this issue). Isfjorden is ice free during winter, and thus no ice algae can develop. Besides, *C. finmarchicus* is not known to feed on ice algae to fuel reproduction (Niehoff et al., 2002 (Disko Bay)). Thus, at that time of the year, the increased energy demand cannot be fueled by food uptake as the spring bloom is weeks ahead. The seasonal pattern in HOAD activities exhibited by *C. finmarchicus* thus differed from that of *C. glacialis* (Freese et al., 2017)

As MDH, CS is a proxy for the overall metabolism, and for this enzyme similar seasonal activity patterns were found for *C. glacialis* in Isfjorden and the colder Billefjorden, supporting the 50% reduction in metabolism in winter as previously estimated by Freese et al. (2017). CS has also been found to be positively correlated to egg production (Kreibich et al., 2008), which is further supported by our study. We found that peak CS activity in *C. finmarchicus* was correlated strongly to Chl *a* concentration, but not in *C. glacialis*. Both *C. glacialis* and *C.*

finmarchicus reproduced over an extended period, but with different timing (Hatlebakk et al., 2022). *Calanus glacialis* males and females appeared in early January and produced eggs from late March on, with a strong peak at the onset of the spring bloom. *Calanus finmarchicus* adults appeared in February-March and started egg production at the onset of the spring bloom in early May and maintained egg production well into the summer (Eide, 2016; Hatlebakk et al., 2022). Thus, the correlation between Chl *a* and CS activity for *C. finmarchicus* likely mirrors that *C. finmarchicus* is an income breeder. *Calanus glacialis*, on the other hand, can start reproduction earlier based on internal reserves (capital breeding) before Chl *a* builds up in the water column of Isfjorden. Thus, when CS increases with egg production, this is not directly linked to the spring bloom in *C. glacialis*, as it is in *C. finmarchicus*.

4.2 Do the Digestive Enzyme Activity Patterns Differ Between the Species?

Lipase and proteinase reached peak activities during the spring bloom, and earlier in *C. glacialis* than *C. finmarchicus*. *Calanus finmarchicus* have been found to be torpid and have reduced gut epithelium during overwintering (Hallberg and Hirche, 1980). Interestingly, specific lipase activities increased much more than proteinase in *C. glacialis*, while the opposite was the case in *C. finmarchicus* in which proteinase increased more than lipase. This may suggest that the two species differ with regard to their potential of using these two dietary enzymes. The higher proteinase activity in *C. finmarchicus* could be part of the adaptation to income-breeder strategy, utilizing food for final oocyte maturation, while *C. glacialis* invests substantial amounts of stored lipids into early egg production (Hirche and Kattner, 1993; Jónasdóttir, 1999; Hatlebakk, 2014). *Calanus finmarchicus* only needs sufficient lipids to sustain basic metabolism through diapause. The higher lipase mobilization in *C. glacialis* could be a physiological adaptation of this arctic species to fully utilize the generally short primary production window to fuel lipid accumulation.

4.3 How do Enzyme Patterns Compare Between Off-Shelf and Fjord?

Sampling off-shelf was focused on the period from March to June 2015, when the N-ICE 2015 campaign allowed for frequent sampling in the area. Then, the community was strongly dominated by *C. finmarchicus* (Hop et al., 2021), and thus, there are too few data for *C. glacialis* to compare with Isfjorden. The MDH and CS activities of *C. finmarchicus* showed similarly low activity levels prior to the bloom and similarly high levels in the bloom as in Isfjorden. Off-shelf, however, enzyme activities were not correlated with day length, which can be explained by the consolidated sea ice with snow on top and concomitantly low irradiances in the water column until late May (Assmy et al., 2017). In the Arctic Ocean, the ice algae and phytoplankton blooms are initiated later, and are of lower magnitude with shorter duration than in Svalbard fjords further south (Leu et al., 2011; Leu et al., 2015), which also was the case in 2015. Off-shelf, food availability increased not before the

beginning of June (Assmy et al., 2017), while in Isfjorden, maximum Chl *a* was already recorded at the end of April (University Centre in Svalbard, 2020). Correspondingly, also the digestive enzyme activities increased later off-shelf, and thus, similar to Isfjorden, corresponded well to the Chl *a* development.

4.4 Food Availability as Cue in Termination of Diapause

There are still questions to resolve regarding mechanisms controlling initiation and termination of diapause in *Calanus*. Based on gene expression patterns and synchronized ascent despite lack of light in their study, Häfker et al. (2018) suggest that an endogenous circannual clock is involved in the termination of diapause. Gene expression was not involved in our study, and unfortunately the community data are not overlapping for the two sites at the time of ascent (Hop et al., 2021, Hatlebakk et al., 2022). However, we note that the ascent of *Calanus* spp. was recorded in early May both off-shelf in 2015 (Hop et al., 2021) and in Isfjorden in 2016 (Hatlebakk et al., 2022), and whilst this is not sufficient to state synchronized ascent across the two sampling sites, it is in line with the hypothesis of Häfker et al. (2018). Even if the ascent to the surface occurred at the same time of the year, the increases in metabolic enzymes as well as digestive enzyme activities were first observed in connection with the increase in Chl *a* concentration. This was the case in early May in Isfjorden, but a month later off-shelf (Assmy et al., 2017). A previous study by Morata and Søreide (2015) has also demonstrated a link between termination of diapause and food availability. In their study, *C. glacialis* CV collected in winter (November) increased respiration when exposed to light. If food was not present, the respiration rates decreased again, while with the presence of food, the respiration remained elevated. Also, feeding during winter initiated an increase in the lipolytic activity of *C. glacialis* CV (Freese et al., 2012). This along with our findings that peak metabolic enzyme activity was measured alongside peak Chl *a* concentrations both in the fjord and off-shelf, indicate that food availability is an important signal for the final step of termination of diapause.

Concluding Remarks

In our study, the seasonal regulation of anabolic and catabolic enzyme activities was overall similar for *C. glacialis* and *C. finmarchicus*, but differed in the variation of digestive enzymes throughout the seasons. *Calanus glacialis* may utilize dietary lipids more efficiently, as reflected by an earlier and higher increase in lipase enzyme activities, while *C. finmarchicus* showed higher increase in proteinase for digestion of proteins. Even small differences in the timing of life-history events can have large impacts on a species success (e.g., Varpe, 2012). A more efficient lipid anabolism of *C. glacialis* may provide an advantage over *C. finmarchicus* in high-Arctic unpredictable environments with short-pulsed primary production regimes. The accumulation of lipids allows for flexibility by providing an

internal energy source, while the strategy of *C. finmarchicus* of relying on external food becomes a limitation with regard to the short-pulsed bloom which is typical for the high Arctic.

The strong seasonality in incoming solar radiation is predictable and will not change with global warming. However, sea ice, snow cover and open leads can strongly modulate the underwater light regime, which impacts the timing of the blooms. The arrival of *Calanus* in surface waters under the ice well before the bloom (Hop et al., 2021), the connection between enzyme activity and Chl *a* as shown in our study, and the increase in catabolism of stored lipids observed in *C. finmarchicus* prior to the spring bloom, indicate that external triggers such as food are important for the final steps of terminating diapause. Thus, while endogenous mechanisms, like a circannual clock, may be important for initiating termination of diapause, there appears to be mechanisms for tuning the timing between the producers and consumers in the planktonic food web to ensure a good match, even with interannual and spatial variability in bloom timing.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MH has conducted sampling, sample analyses, compiling first draft and edited the manuscript based on feedback from co-authors. BN has contributed with initial planning, guidance for enzyme analyses and interpretation of data, and extensive feedback to the manuscript. MC has contributed with molecular analyses, interpretation of this data and feedback to the manuscript. HH and AW have contributed with sampling off-shelf and feedback on the manuscript. GH has contributed with initial planning of the study, guidance for the molecular part of the study and feedback to

the manuscript. JS has contributed with initial planning of the study, sampling in Isfjorden, interpretation of data, compiling the first draft and extensive feedback in the editing process. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.877904/full#supplementary-material>

Supplementary Figure 1 | Weighted mean depth of *Calanus glacialis* and *C. finmarchicus* (A) during the N-ICE 2015 campaign spring 2015 and (B) in Isfjorden from June 2015 to August 2016. Error bars indicate standard deviation.

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